The relationship between the ABO type, Rh factor and the 2D:4D ratio: A cross-sectional study in Ghana

ABSTRACT

Aims: The second-to-fourth digit ratio (2D:4D) is the putative marker of prenatal androgen exposure. The 2D:4D ratiotends to be lower in males than females; however, there are population variabilities. Previous studies have suggested a possible association between the ABO and Rhesus blood group systems with the 2D:4D ratio. This observation has not been tested in a Ghanaian population.

Study design: The study was cross-sectional

Place and Duration of study: The study was conducted in Tamale between May and June 2021.

Methodology: There were 206 participantscomprising males (n=94) and females (n=112), aged between 18 to 32 years. The right digit ratio (2D:4DR), the left digit ratio (2D:4DL) and the right-left difference in digit ratio (Dr-l) were measured using computer-assisted analysis. The ABO blood type and the Rh factor were determined using monoclonal antibodies. The effect sizes were reported as either Hedge's *g* for a*t*-test or partial Eta-squared (η_p^2)for the one-way ANOVA test.

Results: Males' 2D:4DL was lower than females with a small effect size (P= 0.033, *g*: 0.28). The differences in digit ratios by the ABO blood type had small to medium effect sizesin both males and females (P ≥ 0.050,η_p^2: 0.00-.08).In females, the 2D:4DR of participants with Rh- was higher while the 2D:4DL and the Dr-l were lowerthan participants withthe Rh+ with medium to large effect sizes (P< 0.050, *g:* 0.56, 0.84, 2.72 respectively). In males, the 2D:4DL and the Dr-l were higher in participants with Rh+than the Rh- participants with large effect sizes (P< 0.050, *g:* 1.00, 2.52 respectively).

Conclusion: The expression of the Rhesus factor may affect the expression of the 2D:4D ratio in the study population.

Keywords: 2D:4D ratio, Prenatal Androgens, ABO blood type, Rh factor, Ghana

1. INTRODUCTION

The ratio of the length of the fore-finger to the length of the ring-finger is termed the 2D:4D ratio. It has been suggested that the 2D:4D ratio is a putative marker of prenatal androgen exposure. The ratio is sexually dimorphic with males showing lower ratios than females (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2004; Manning, Scutt, Wilson, & Lewis-Jones, 1998). Previous twin and familial studies have demonstrated that the 2D:4D ratio is heritable due to the observation of significant additive genetic effects(Gobrogge, Breedlove, & Klump, 2008; Richards, Bellin, & Davies, 2017). Also, the 2D:4D ratio is influenced by assortative mating (Voracek, Dressler, & Manning, 2007). The heritability of the 2D:4D ratio may be complex as several genes may be involved. However, the Homeobox and the androgen receptor genes have been suggested to play a role in the heritability of the 2D:4D ratio. Recent advances in molecular biology have also identified novel gene loci that may be associated with the 2D:4D ratio (Warrington et al., 2018).

The ABO and the Rh are the two major blood group systemsfrom the over 30 known blood groups systems in humans(Flegel, 2011). The gene that controls the expression of the ABO system is located on chromosome 9 with locus at q34.2 while the genes for Rh are located at p36.11 on the short arm of chromosome 1 (Voracek, 2008). The ABO system has four main phenotypes; A, B, AB and O while the Rh system has several phenotypes but the RhDisthe most immunogenic (Doku, Agbozo, Annor, Kisseh, & Owusu, 2019).Some genes that are associated with the 2D:4D ratio may be located around the vicinity of the ABO and

Rh loci and may be inherited together or there may be some level of pleiotropismbetween these genes (Voracek, 2008; Warrington et al., 2018).

Previous studies have attempted to draw an association between the 2D:4D ratioand many human phenotypes(Butovskaya et al., 2015; Stibbard-Hawkes, 2020). However, the work of Voracek (2008) has been the single most significant study that sought to determine an association between the 2D:4D ratio and human blood group systems. This study sought to determine the association between the ABO system, the Rhfactor and the 2D:4D ratio in a Ghanaian population because there are variabilities in the 2D:4D ratio and the distribution of human blood group systems within and between populations(Doku et al., 2019; Manning, 2002).

2.0 MATERIALS AND METHODS

2.1 Study design and settings

The study was cross-sectional from May to June 2021 at the University for Development Studies (UDS), Tamale. UDS is a multicampus, multidiscipline and multinational university that is located in the Northern region of Ghana. The Tamale campus is the largest and a host to academic programs in the fields of Medical Laboratory Sciences, Nursing, Nutritional Sciences, Medical Imaging, Pharmaceutical Sciences, Medicine and Education(UDS, 2020).

2.2 Participants

The study involved 206 adults (males: 94 and females: 112) within the age range of 18 to 32 years. The target study population was stratified by sex before a nonprobability sampling technique was used to select the study participants. The participants were devoid of fractures or deformities to their fingers or upper extremities. Participation in the study was voluntary and no restrictionswere placed on a participant'scultural group, the program of study or religious affiliation.

2.3 Variables

The 2D:4D ratios and their derivatives (M2D:4D and Dr-l) were the independent variables. The ABO type and Rh factor of the participants were the dependent variables. The participants' age, cultural group and body mass index (BMI) were the additional variables.

2.4 Data collection

2.4.1 Digit measurements

The digit or finger lengths of all the participants were measured using a computer-assisted program. The participant was asked to remove all objects such as rings from their fingers before the palmar surface of each hand was placed on the flatbed surface of a Hp 2620 series Desk jet scanner (HP Inc. CA 94304 United States). The participant's second to fifth fingers wereheld parallel and the tip of the middle finger aligned with the wrist and elbow(Allaway, Bloski, Pierson, & Lujan, 2009). The palm and fingers were then scanned, together with the participant's study unique identifier, at a resolution of 150 dpi. The scanned images were then exported to GIMP (v 2.10.22), an image manipulation program (www.gimp.org). Finger lengths were measured from the mid-point of the most proximal flexion crease to the tip of each finger using a mouse-assisted calliper. Measurements were taken twice by one observer at a week's interval. The intraclass correlation coefficients (two-way mixed, single measures with absolute agreement) were found to be 0.98 and 0.97 respectively for the right (2D:4DR) and the left (2D:4DL) digit ratios. The two measurements were then averaged to obtain the final value. The mean of the left and right 2D:4D ratio was calculated (M2D:4D) and also the right-left difference or directional asymmetry(Dr-l).

2.4.2 Blood sample collection and typing

Venous blood was collected into an EDTA anticoagulant tube. The tubes were gently inverted about eight times to ensure proper mixing of the blood with the anticoagulant. The blood samples were centrifuged at 1500 rpm for five minutes to obtain the red cells

(Canizalez-Román et al., 2018). The red cells were washed three times with saline before typing by agglutination using ant-A, anti-B and anti-D monoclonal antibodies (Immucor Inc., Norcross, GA, USA).

2.5 Statistical Analysis

The data were collected onto an Excel spreadsheet (RRID:SCR_016137) before statistical analysis in SPSS (v23) (RRID:SCR_019096) and GraphPad Prism (v8)(RRID:SCR_002798). The continuous data were checked for outliers and normality using the ROUT test and the Kolmogorov-Smirnov test respectively. Descriptive statistics were performed and parametric variables were presented as mean ± SD and frequency (%) for categorical variables. The differences between male and female variables were determined using the student t-test (unpaired, 2-tailed). For the categorical variables, the sex variable was dummy coded (female=zero, male=one) before logistic regression analysis. The ABO type wasdummy coded (O=zero, A=one, B=two and AB=three) and also the Rh factor (dummy coded Rh+=0 and Rh- =1). The differences in means of the digit ratios by the participant ABO blood types were compared using the one-way ANOVA test followed with the Posthoc test (Bonferroni). The mean digit ratios by participants' Rh factor were compared using the student t-test (unpaired, 2-tailed). The Hedge's *g* method was used to estimate the effect sizes for the t-test due to the differences in male and female sample sizes while the partial Eta-squared (η_p^2) was used to estimate the effect sizes in the one-way ANOVA analysis(Fritz, Morris, & Richler, 2012).

To determine the relationship between digit ratios and the distribution of the ABO type and Rh factor among the participants, digit ratios ≤ mean were regarded as low (dummy coded as zero) and digit ratios > mean were regarded as high (dummy coded as one). Participants with a right-left difference in digit ratio (Dr-l) < 0.00 were regarded as having a more leftward asymmetry (dummy coded as zero) while those with Dr-l ≥ 0.00 were considered as having a more rightward asymmetry (dummy coded as one). Logistic regression analysis was then performed to establish associations. The ABO type,the Rh factor and the cultural group variables were entered simultaneously into the same model for all logistic regression analyses because of possible pleiotropic effects and cultural variabilities. The results were then reported as adjusted odds ratios (AOR) with their 95% confidence intervals (CI). All analyses were 2-tailed and a probability value < .050, was considered statistically significant.

4.0 RESULTS

4.1 The general characteristics of the study population

The general characteristics of the study population are summarized in Table 1. The study involved 206 participants with females constituting 54.4% (112/206) and males, 45.6% (94/206). The study participants were aged between 18 to 32 years with a mean ± SD age of 22.6±2.61 years. The ABO type O was the most common (48.4%) and the AB type was the least common (4.4%). Also, the Rh+ formed the majority (89.4%) while the Rh- constituted the remainder (10.4%).

Table 1. General characteristics of the study population

Variable	Statistic
Age(years)	22.6±2.61
Sex	
Female	112(54.4)
Male	94(45.6)
Cultural group	
Mole-Dagomba	61(29.7)
Akan	63(30.4)
Others	82(39.9)
ABO type	
O	100(48.4)
A	43(20.9)
B	55(26.4)
AB	8(4.4)
Rh type	
Rh+	185(89.6)
Rh-	21(10.4)
BMI (Kg/m2)	22.0±2.95
2D:4DR	0.94±0.034
2D:4DL	0.94±0.036
M2D:4D	0.94±0.033
Dr-l	-0.001±0.027

Note. The results were presented as mean ± SD for continuous variables and frequency (%) for categorical variables.

4.2 Comparison of male and female variables

From Table 2, the mean 2D:4DL of males was significantly lower than females (P=.033), but the effect size was small (*g*= 0.28). However, there were no significant differences in the distribution of the ABO type and Rhfactor between the male and female participants.

Table 2. Comparison of male and female blood types and anthropometric variables

Variable	Female	Male	AOR (95%CI)/P-value	*g*
ABO type				
O	57(57.0)	43(43.0)	1	
A	21(48.7)	22(51.3)	1.400(0.646-3.035)	
B	34(61.5)	21(38.5)	0.823(0.374-1.815)	
AB	4(50.0)	4(50.0)	1.367(0.316-5.919)	
Rh type				
Rh+	103(55.5)	82(44.5)	1	
Rh-	12(58.8)	9(41.2)	0.830(0.295-2.334)	
2D:4DR	0.94±0.035	0.93±0.033	0.103	0.293
2D:4DL	0.94±0.036	0.93±0.034	0.033	0.285
M2D:4D	0.94±0.034	0.94±0.031	0.035	0.000
Dr-l	-0.003±0.026	-0.000±0.027	0.460	0.113

4.3 Differences in digit ratio by the ABO type

The digit ratios, their mean and the difference were stratified by the ABO type in both females and males (Table 3). Although there were no significant differences in digit ratios by the ABO blood type, individuals with the AB type had the highest left, right and mean 2D:4D ratio while individuals with type A had the highest asymmetry (Dr-l) among females. However, in males, participants with the ABO type B had the lowest 2D:4D ratio while those with type AB had the highest M2D:4D. The ABO type O had the lowest asymmetry (Dr-l) among males. The effect sizes were small to medium (η_p^2: 0.00 - 0.08)

Table 3. Comparison of digit ratios by ABO blood type in males and females

Variable	O	A	B	AB	P-value	η_p^2
Female						
2D:4DR	0.94±0.035	0.94±0.032	0.95±0.035	0.98±0.034	0.062	0.081
2D:4DL	0.94±0.037	0.95±0.035	0.95±0.038	0.98±0.037	0.262	0.045
M2D:4D	0.94±0.035	0.94±0.030	0.95±0.033	0.98±0.033	0.110	0.067
Dr-l	-0.004±0.024	0.009±0.030	0.003±0.029	0.003±0.010	0.538	0.025
Male						
2D:4DR	0.94±0.029	0.94±0.032	0.92±0.026	0.94±0.084	0.384	0.044
2D:4DL	0.94±0.033	0.94±0.030	0.92±0.033	0.94±0.028	0.155	0.074
M2D:4D	0.94±0.029	0.94±0.030	0.92±0.026	0.95±0.056	0.216	0.063
Dr-l	-0.001±0.024	-0.002±0.018	0.003±0.032	-0.003±0.062	0.959	0.004

Note. Results were presented as mean ± SD. The one-way ANOVA test with Post hoc (Bonferroni) analysis was performed to determine the differences between the means. The effect sizes were presented as partial Eta-squared (η_p^2): small ($0.01<\eta_p^2\leq0.06$), moderate ($0.06<\eta_p^2\leq0.14$), large ($\eta_p^2>0.14$).

4.4 Differences in digit ratio by the Rh factor

Digit ratios were compared by the Rhfactorin both males and females (Table 4). Among the females, the 2D:4DR was significantly lower while the 2D:4L was significantly higher in those with Rh+, relative to the Rh-,with medium to large effect size (P<0.050, *g*: 0.56 – 2.72). Also, the males with Rh+ had significantly higher 2D:4DL as compared to their counterparts with the Rh-with a large effect size (P<.050, *g*: 1.00 – 2.52).

Table 4. Comparison of digit ratios by Rh blood type in males and females

Variable	Rh+	Rh-	P-value	*g*
Female				
2D:4DR	0.93±0.036	0.95±0.033	0.010	0.560
2D:4DL	0.96±0.036	0.93±0.033	<0.001	0.840
M2D:4D	0.95±0.036	0.94±0.032	0.502	0.281
Dr-l	-0.025±0.015	0.016±0.016	<0.001	2.715
Male				
2D:4DR	0.92±0.029	0.94±0.034	0.250	0.678
2D:4DL	0.95±0.030	0.92±0.031	<0.001	0.997
M2D:4D	0.94±0.029	0.93±0.032	0.238	0.342
Dr-l	-0.026±0.017	0.017±0.018	<0.001	2.516

Note. Results were presented as mean ± SD. The differences between the means were determined using the student t-test (unpaired, 2-tailed). The effect sizes were presented as Hedge's *g*: similar (*g*<0.20), small (0.20≤*g*<0.50), moderate (0.50≤*g*<0.80), large (*g*≥ 0.80).

4.5 The association between the 2D:4D ratio and blood type

To determine the association between prenatal androgen exposure (as indexed by the 2D:4D ratio) and the distribution of the ABO type and Rhfactor, the participants' blood types

were compared by low (≤ mean) and high (> mean) digit ratios in both males and females (Table 5). Both the ABO type and the Rhfactor were entered in the same logistic regression model. No significant associations between the 2D:4D ratios and the ABO type or the Rhfactor were observed.

Table 5. The association between the 2D:4D ratio and the distribution of the ABO and Rh blood types

Variable	Female				Male			
	≤Mean	>Mean	AOR (95%CI)	P-value	≤Mean	>Mean	AOR (95%CI)	P-value
2D:4DR								
O	37(59.1)	20(40.9)	1		21(48.5)	22(51.5)	1	
A	11(52.6)	10(47.4)	1.295(0.438-3.829)	0.641	9(40.0)	13(60.0)	1.472(0.473-4.577)	0.504
B	14(41.7)	20(58.3)	2.037(0.738-5.619)	0.170	15(73.3)	6(26.7)	0.351(0.092-1.337)	0.125
AB	1(25.0)	3(75.0)	4.279(0.409-44.787)	0.225	3(75.0)	1(25.0)	0.286(0.026-3.155)	0.307
Rh+	53(51.9)	50(48.1)	1		44(53.8)	38(46.2)	1	
Rh-	6(50.0)	6(50.0)	1.101(0.283-4.284)	0.890	4(42.9)	5(57.1)	1.786(0.337-9.454)	0.495
2D:4DL								
O	31(54.5)	26(45.5)	1		16(36.4)	27(63.6)	1	
A	11(52.6)	10(47.4)	1.080(0.367-3.180)	0.889	8(35.0)	14(65.0)	1.158(0.357-3.757)	0.807
B	16(45.8)	18(54.2)	1.419(0.520-3.869)	0.494	11(53.3)	10(46.7)	0.525(0.149-1.849)	0.316
AB	1(25.0)	3(75.0)	3.597(0.344-37.593)	0.285	2(50.0)	2(50.0)	0.456(0.050-4.158)	0.486
Rh+	52(50.6)	51(49.4)			35(43.1)	47(56.9)	1	
Rh-	6(50.0)	6(50.0)	1.007(0.261-3.885)	0.992	1(14.3)	8(85.7)	4.966(0.537-45.933)	0.158
M2D:4D								
O	30(52.3)	27(47.7)	1		21(48.5)	22(51.5)	1	
A	12(57.9)	9(42.1)	0.798(0.269-2.367)	0.684	9(40.0)	13(60.0)	1.604(0.507-5.078)	0.507
B	11(33.3)	23(66.7)	2.185(0.773-6.177)	0.140	14(66.7)	7(33.3)	0.496(0.133-1.847)	0.133
AB	1(25.0)	3(75.0)	3.3(0.316-34.499)	0.319	2(50.0)	2(50.0)	0.731(0.077-6.925)	0.077
Rh+	48(46.9)	55(53.1)	1		44(53.8)	38(46.2)	1	
Rh-	6(50.0)	6(50.0)	0.969(0.248-3.777)	0.963	1(14.3)	8(85.7)	7.725(0.844-70.670)	0.844

Note. Results were presented as frequency (%). Male and female blood types were compared by lower (≤ mean) and higher (> mean) digit ratios. Both the ABO and the RhD blood types were entered simultaneously into the same model for each digit ratio and the adjusted odds ratio (AOR) reported along with their 95% confidence interval (CI).

4.6 The association between digit ratio asymmetry and blood type

The ABO and Rh blood types in both males and females were compared by more leftward (Dr-l < 0.00) and more rightward (Dr-l ≥ 0.00) asymmetry (Table 6). No significant associations were observed between the Dr-l and the distribution of the ABO type or the Rhfactor.

Table 6. The association between digit ratio asymmetry and the blood group type

Variable	<0.00	≥0.00	AOR (95%CI)	P-value
Female				
O	26(45.5)	31(54.5)	1	0.603
A	12(57.9)	9(42.1)	0.620(0.208-1.850)	0.391
B	14(41.7)	20(58.3)	1.115(0.405-3.070)	0.834
AB	1(25.0)	3(75.0)	2.779(0.259-29.843)	0.399
Rh+	46(44.4)	57(55.6)	1	
Rh-	7(60.0)	5(40.0)	0.527(0.132-2.106)	0.365
Male				
O	18(42.4)	25(57.6)	1	
A	8(35.0)	14(65.0)	1.312(0.412-4.177)	0.646
B	7(33.3)	14(66.7)	1.428(0.396-5.153)	0.586
AB	3(75.0)	1(25.0)	0.260(0.024-2.818)	0.268
Rh+	32(38.5)	50(61.5)	1	
Rh-	5(57.1)	4(42.9)	0.549(0.108-2.799)	0.470

Note. Results are presented as frequency (%). The ABO and Rh blood types were entered in the same logistic regression model to obtain the adjusted odds ratios (AOR) and confidence intervals (CI).

5.0 DISCUSSION

The study sought to determine the association between prenatal androgen exposure (as indexed by the 2D:4D ratio) and the ABO type or Rh factor. It was observed that the 2D:4DL of males was significantly lower than females. There were no significant differences in digit ratio by the ABO type but the effect sizes were small to medium. However, females with the ABO type AB and type B had the highest digit ratio and asymmetry (Dr-l) respectively.Also, males with ABO type B had the lowest 2D:4D ratio while those with type O had the lowest asymmetry (Dr-l).But females with Rh- had significantly higher 2D:4DR while those with Rh+ had significantly higher 2D:4DL and Dr-l with medium to large effect sizes, when compared to those with Rh-. Moreover, males with Rh+ had significantly higher 2D:4DL and Dr-l as compared to those with Rh- with large effect sizes. But there was no significant association between the 2D:4D ratio and the distribution of the ABO type or the Rhfactor in the study population.

Studies regarding the association between the 2D:4D ratio and blood type are rare. As of the time of writing, the work of Voracek (2008)was the most significant study on the subject. The study of Voracek (2008) involved two separate samples, I and II: Sample Iconsisted of 200 males and 200 females while sample II consisted of 395 males and 478 females, drawn from theAustrian population. For both samples, males had a lower 2D:4D ratio in both hands but a higher asymmetry (Dr-l) for sample II. Voracek (2008) observed that the differences in the 2D:4D ratio by the ABO or Rh factor were small and not significant inboth samples.

There have been many suggestions aimed at the possible explanations for these observed differences in digit ratios by ABO and Rh blood systems. There is evidence from familial studies that the 2D:4D ratiois heritable in humans, just like the ABO and the Rh systems(Richards et al., 2017). The genes that regulate the expression of the 2D:4D ratios may be located at loci that are in proximity to genes that control the expression of the ABO and Rh blood systems. For the ABO system, this would be on the long arm of chromosome

9, in the vicinity of the gene loci, q34.2 while for the Rh factor, this would be on the short arm of chromosome 1, around positionp36.11 gene loci. Another possible explanation is the seeming pleiotropic effects of either the genes controlling the 2D:4D ratio on the genes for the ABO and Rh system or vice versa (Voracek, 2008). The androgen receptor gene (AR), located on the X-chromosome, have been suggested to be associated with the 2D:4D ratio. Polymorphisms in the AR gene (CAG repeats) have been associated with androgen insensitivity and differences in the 2D:4D ratio, although this is not a universal observation (Butovskaya et al., 2012; Zhang et al., 2020). However, the AR gene has pleiotropic effects on other genes whose expression or suppression may be associated with the expression of the 2D:4D ratio (Aurilio et al., 2020; Q. Li et al., 2019).

In one of the largest meta-analytic genetic association studies regarding the 2D:4D ratio, 9 novel and two previously known loci were associatedwith the 2D:4D ratio expression in humans. In total, the 11 genes accounted for about 3.8% of the variance in the mean 2D:4D ratio(Warrington et al., 2018). The Glis Family Zinc Finger 1 (GLIS1) gene (locus; 1p32.3) which is located on the same chromosome as the Rh factor (chromosome 1), plays an important role in embryogenesis by controlling gene expression at different stages in the process (L. Li et al., 2020). The Ephrin A1 (EFNA1) gene (locus 1q21-q22), also located on chromosome 1, has been shown to encode for a protein that plays a role in the mediation of developmental events (Warrington et al., 2018).Other significant genes, that are associated with the 2D:4D ratio but are not located on the same chromosome as the ABO or Rh genes, but may have a pleiotropic effect were also identified. The gene for Rh-associated glycoprotein (RhAG), denoted as RHAG, which is vital for the expression of the Rh polypeptide is located on chromosome 6 (6p11–p21) (Flegel, 2011).The Lipid droplet-associated hydrolase (LDAH), a gene that can be found around locus, 2p24.1, may be involved in steroidogenesis as it mobilizes cholesterol which could be converted into testosterone (Goo, Son, Kreienberg, & Paul, 2014). Testosterone plays a marked role in the expression of the 2D:4D ratio. Individuals diagnosed with congenital adrenal hyperplasia (CAH), due to 21-hydroxylase deficiency, tend to have androgenized or masculinized 2D:4D ratios while those with androgen insensitivity orKlinefelter's syndromes tend to have feminized digit ratios although the effect sizes may be small (Richards et al., 2020). The Obg-like ATPase 1(OLA1) is a gene that has a locus at2q31.1. Animal models that lacked the OAL1 gene were observed to have delayed development characterized by stunted growth and immature organs (Ding et al., 2016). The 7p14.1 locus, which is linked to the Zinc finger protein (GLI3) has been implicated in craniofacial and limb deformities such as polydactyly and brachydactyly(Al-Qattan, Shamseldin, Salih, & Alkuraya, 2017). Similarly, the 11q24.1-q24.3 locus, which is intergenic between the Friend leukaemia integration 1 transcription factor (FLI1) and ETS1 gene has also been suggested to play a role in preaxial polydactyly (Lettice et al., 2012). A signal at the locus, 16q12.1, in the vicinity of the spalt-like1 (SALL1) gene on chromosome 16, and SALL3 (18q23) have been associated with skeletal abnormalities including hand and genital malformations (Kohlhase et al., 1999). There was some level of association between HOXD12 and the 2D:4D ratio. HOXD12 belong to the Homeobox family of genes that play a vital role in morphogenesis, including the development of the limbs and genitalia (Warrington et al., 2018). There are no direct shreds of evidence that these genes are also associated with the ABO or Rh systems, but there could be some possible pleiotropic interactions between these genes controlling both systems or these genes maybe involved either directly or remotely, in steroidogenesis during human foetal development (Voracek, 2008).

The current study has many strengths; to the best of our knowledge, this study is the first to be conducted in Ghana regarding digit ratios and the ABO or the Rh blood group systems. This is significant owing to the variabilities in the 2D:4D ratio and the distribution of blood group systems in human populations. Unlike Voracek (2008), results of blood type were obtained from laboratoryanalysisand not from self-reported data which could have accounted for the differences in outcomes betweenthe two studies. In this study, digit ratios were

measured by computer-assisted analysis which is more precise compared to measurements from photocopies, physical measurements and printed scanned images (Allaway et al., 2009). Also, both the ABO and the Rh variables were entered simultaneously into the same regression model to reduce confounding in case there are any pleiotropic effects between genes controlling both systems. Moreover, aside from P-values, effect sizes (Hedge's *g* and partial Eta-squared) were presented to add clarity to the observed differences. However, some limitations exist in the current study: there was neither genetic studies regarding the inheritance of the 2D:4D ratio, ABO or the Rh factor nor participants detailed data regarding theirancestry(Voracek, 2008). Also, the sample size was smaller, relative to a previous study (Voracek, 2008), which may not allow for the generalization of the findings. In conclusion, there are significant differences in the 2D:4D ratio between the Rh+ and Rh- participants. We, however, recommend further studies with a larger sample size in which the minor blood group systems will also be studied. Further studies should also include genetic and detailed ancestral studies of the participants.

7.0 CONCLUSION

The second-to-fourth digit ratio, a marker of prenatal hormone exposure may be associated with blood type, particularly the Rhesus blood type. This, however, requires further studies to validate the observations of the current study.

ETHICAL APPROVAL

The study followed all guidelines regarding human studies as stipulated in the 1964 Declaration of Helsinki and its later amendments. The study was approved by the institutional review board of the University for Development Studies, Tamale. All participants gave their written informed consent before the study.

LIST OF ABBREVIATIONS

2D:4D	Second-to-fourth digit ratio
AR	Androgen receptor
BMI	Body Mass Index
CI	Confidence Interval
EFNA1	Ephrin A1
FLI1	Friend leukaemia integration 1 transcription factor
GLI3	Zinc finger protein
GLIS1	Glis Family Zinc Finger 1
HOX	Homeo Box
L	Left
LDAH	Lipid droplet-associated hydrolase
LIN28B	Lin-28 homolog B
M	Mean
OLA1	Obg-like ATPase 1
R	Right
SALL1	Spalt-like1
UDS	University for Development Studies

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